

Effects of Light and Water Availability on the Performance of Hemlock Woolly Adelgid (Hemiptera: Adelgidae)

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ABSTRACT Eastern hemlock (*Tsuga canadensis* (L.) Carriere) is a dominant shade-tolerant tree in northeastern United States that has been declining since the arrival of the hemlock woolly adelgid (*Adelges tsugae* Anand). Determining where *A. tsugae* settles under different abiotic conditions is important in understanding the insect's expansion. Resource availability such as light and water can affect herbivore selectivity and damage. We examined how *A. tsugae* settlement and survival were affected by differences in light intensity and water availability, and how adelgid affected tree performance growing in these different abiotic treatments. In a greenhouse at the University of Rhode Island, we conducted an experiment in which the factors light (full-sun, shaded), water (water-stressed, watered), and adelgid (infested, insect-free) were fully crossed for a total of eight treatments (20 two-year-old hemlock saplings per treatment). We measured photosynthesis, transpiration, water potential, relative water content, adelgid density, and survival throughout the experiment. Adelgid settlement was higher on the old-growth foliage of shaded and water-stressed trees, but their survival was not altered by foliage age or either abiotic factor. The trees responded more to the light treatments than the water treatments. Light treatments caused a difference in relative water content, photosynthetic rate, transpiration, and water potential; however, water availability did not alter these variables. Adelgid did not enhance the impact of these abiotic treatments. Further studies are needed to get a better understanding of how these abiotic factors impact adelgid densities and tree health, and to determine why adelgid settlement was higher in the shaded treatments.

KEY WORDS *Adelges tsugae*, *Tsuga canadensis*, plant–insect interaction

Introduction

The hemlock woolly adelgid (*Adelges tsugae* Anand) is an invasive species that poses a major threat to eastern hemlock (*Tsuga canadensis* (L.) Carriere) on the east coast of the United States. The adelgid was introduced to eastern Virginia in the early 1950s from Japan and spread rapidly northward, reaching New England by 1985 (McClure 1989a). It completes two generations per year and is obligately asexual in its invaded range (McClure 1989b), two factors that have helped it spread rapidly through the Northeast. In its invaded range, the adelgid feeds exclusively on eastern and Carolina hemlock (*T. carolinensis*) and can kill mature trees in as little as four years (McClure 1991), although some trees can survive for >10 yr (Orwig et al. 2002). In its juvenile “crawler” phase, *A. tsugae* crawlers can move within vegetation or be passively dispersed among trees by wind, birds, or other vectors (McClure 1989b, Turner et al. 2011). Once it locates a suitable feeding site at the base of a hemlock needle, the crawler inserts its stylet bundle and begins feeding on xylem ray parenchyma cells; it will stay in this feeding site for the remainder of its life (Young et al. 1995). The adelgid is now found throughout New England, ranging as far south as Georgia, and poses a significant threat to hemlocks in this

region (Orwig et al. 2012). Hemlocks are considered “foundation species” in eastern forests, and their loss will greatly impact both terrestrial and aquatic ecosystems as well as ecosystem processes such as carbon sequestration and nutrient cycling (Ellison et al. 2005).

Researchers have recently begun addressing the mechanism(s) underlying the adelgid's rapid and lethal impact on hemlock trees. The adelgid has been shown to cause a systemic hypersensitive response, a defensive response linked to plant stress, in hemlock trees (Radville et al. 2011). The hypersensitive response, a common response to pathogens and sessile insect herbivores, kills the tissue surrounding the feeding and infection site by starving it of water and nutrients (Heath 2000). Perhaps as a result, *A. tsugae*-infested trees have a greater number of false growth rings, bands of thick-walled latewood indicative of water stress, than uninfested trees (Gonda-King et al. 2012). The adelgid is also known to affect other water-related parameters in eastern hemlock, and to reduce overall tree water use by >40% (Domec et al. 2013). Infestation by *A. tsugae* also increases amino acid concentrations at the site of the herbivore's feeding: the largest increase is in proline, an amino acid that acts as an osmoprotectant (Gómez et al. 2012). Furthermore, *A. tsugae* alters plant processes by decreasing stomatal conductance and photosynthesis (Gonda-King et al. 2014).

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Despite our improved understanding of the *A. tsugae*–hemlock interaction, the impact of abiotic factors such as light and water availability on this relationship has not been assessed. There is some evidence that water stress renders hemlocks more susceptible to *A. tsugae* damage (Souto et al. 1996) and that trees decline more quickly on xeric versus mesic sites (Preisser et al. 2008). During a series of stand-level surveys, we have also noticed that understory hemlocks in high-shade conditions appear to decline more quickly than do hemlocks growing in full sunlight (E. Preisser, personal observation). One explanation for this result is that plants experiencing stress may become more susceptible to herbivores (the plant stress hypothesis; White 1984). Conversely, healthy and unstressed plants may provide high-quality resources necessary for optimal herbivore growth (the plant vigor hypothesis; Price 1991).

We report the results of work testing how altered light and water availability affected eastern hemlock, the progrediens generation of *A. tsugae*, and the *A. tsugae*–hemlock interaction. Specifically, we assessed the response of greenhouse-grown hemlock saplings in a $2 \times 2 \times 2$ factorial experiment that crossed light (shade vs full-sun) and water (drought vs watered) with progrediens-generation adelgids (presence vs absence). Because insects such as *A. tsugae* are thought to do better on stressed trees, we hypothesized that *A. tsugae* would settle better and survive longer on shaded, water-stressed trees. Because *A. tsugae* has also been shown to decrease hemlock photosynthesis and stomatal conductance while increasing water potential, we further hypothesized that the presence of *A. tsugae* would exacerbate the impact of abiotic stress on eastern hemlock physiology.

Materials and Methods

In February 2013, we purchased 165 two-year-old uninfested *T. canadensis* saplings (~0.5 m in height) from Van Pines Nursery (West Olive, MI). On arrival, each sapling was individually planted into a 3.8-liter plastic pot with potting soil (Sun Gro Metro-Mix 830; Agawam MA) and watered. The potted trees were placed in a greenhouse at the University of Rhode Island (Kingston, RI) in a grid with 0.5-m spacing; trees were rotated to a new randomly chosen position within the grid every two weeks. Each tree was fertilized two weeks posttransplantation with 175 ppm of 20-10-20 peat lite special. The fertilizer was applied using a 5-s spray from a Dosatron D14MZ2 direct injection proportioner. After three weeks, the trees were inspected and five unhealthy trees were removed; each of the remaining 160 trees appeared healthy and had begun to put on new growth. Forty of the 160 remaining trees were then randomly assigned to one of four treatments: watered–full-sun, watered–shaded, water-stressed–full-sun, and water-stressed–shaded. Within each 40-tree group, 20 randomly selected trees were assigned to an adelgid-infestation treatment and the other 20 trees were assigned to an adelgid-free control. This produced a total of eight 20-tree groups.

To create the watered and water-stressed treatments, the soil moisture in each pot was measured every other

day using an ML2× soil moisture probe and an HHI readout (Dynamax Inc., Austin, TX) accurate to $\pm 1\%$. After soil moisture levels in the 160 pots were measured, data from the 20 trees in each of the four watered treatments and 20 trees from each of the four water-stressed treatments was averaged to generate a mean soil moisture in the four watered and four water-stressed treatments. When average soil moisture in one of the watered treatments dropped below 30%, all 20 trees in that treatment were watered to field capacity by slowly watering each plant until water dripped quickly out of the bottom of the pot. When average soil moisture in one of the water-stressed treatments dropped below 15%, all 20 trees in that treatment were also watered to field capacity as described above. In the course of the experiment, trees in the adelgid-present versus -absent treatments did not differ in their watering regime.

To create the light treatments, trees in the full-sun treatment were individually covered with a 0.2 m³ bag of 10% shade cloth (ShadeClothStore, Libertyville, IL). Trees in the shaded treatment were individually covered with a 0.2 m³ bag of 90% shade cloth. To minimize the contact between the bags and the trees, we inserted three ~0.6 m bamboo stakes at the edge of each pot (at 0, 120, and 240 radial degrees) before placing the bags on the trees; each tree's bag rested on the bamboo stakes rather than the foliage.

After six weeks of exposure to the watered–water-stressed and full-sun–shade treatments, the 40 trees per treatment were split equally into adelgid-infested and uninfested treatments (20 trees per treatment). Crossing the watered–water-stressed and full-sun–shade treatments with an adelgid infested–uninfested treatment created a total of eight 20-tree treatments.

Adelgids were applied to each of the trees in the infested treatments using adelgid-infested foliage collected from Greenfield, MA. Foliage was attached to each tree using standard protocols (Butin et al. 2007); briefly, we selected branches ~15 cm in length from naturally growing hemlocks that were infested with adelgids. We only collected branches that contained wool-bearing adelgids on >50% of the 15-cm segment. To control for the disturbance associated with applying the foliage, uninfested foliage was applied to each tree in the uninfested treatments using pest-free foliage collected from Barre, VT. When the inoculations were checked four days later, few adelgid crawlers were visible; to ensure that the experimental trees were fully infested, more adelgid-infested foliage was collected from the University of Rhode Island campus (Kingston, RI). After checking the foliage to ensure that no non-adelgid pests were present, a single branch was added to each of the trees in the infested treatment. Following this round of inoculations, first-instar crawlers were clearly visible moving and settling on the trees.

Plant Measurements. We measured growth, water potential, and gas exchange parameters on each of the 160 experimental trees. To account for any initial differences in hemlock seedling size, we measured the

height of every tree from the soil surface to the tip of the tallest branch. These initial measurements were used as covariates during analysis. We used a Scholander pressure bomb to make monthly water-potential measurements on each tree from April to July. The April measurement was taken prior to adelgid inoculations, while the May–June–July measurements were taken following the inoculations. Each measurement took two to four consecutive days depending on the number of trees. Two hours before sunrise, two clippings were taken from each tree. One clipping was ~6 cm and included both old and new growth; the other clipping was ~4 cm and included only new growth. Approximately 0.05 g of old-growth needles were removed from the base of each 6-cm clipping. After being weighed, the old-growth needles were put into a coin envelope, placed into a 60° C drying oven for 1 wk, and reweighed. Relative water content was determined by subtracting dry weight from wet weight and dividing by the wet weight. We took data in April, May, June, and July; for the May sampling experimental error precluded analysis of old growth samples. The same procedure was followed using new-growth needles from the 4-cm cutting to determine their relative water content. To take water-potential measurements, the stem of each 6-cm clipping was cut to reveal fresh vascular cambium and individually placed into a pressure bomb. Nitrogen gas was added to the chamber; when fluid emerged from the xylem, the pressure in bars was recorded, and then converted to MPa to get the water potential for the cutting.

At the same time we took monthly water-potential measurements, we also took gas exchange measurements using a CIRAS-2 photosynthesis meter (PP Systems, Amesbury, MA). We simultaneously measured photosynthesis, transpiration, and stomatal conductance between 1 h after sunrise and 11:30 a.m., with the CIRAS set as follows: ambient light, CO₂ reference = 390 ppm, H₂O reference = 100 ppm. Three measurements were taken per branch per tree and used to generate a mean value for each parameter. Because the needles in the CIRAS cuvette did not fill the entire chamber, we took a picture of each branch while inside the cuvette and calculated the needle area using ImageJ (Java Systems) in order to get the actual gas exchange measurements. Because the old- and new-growth foliage grew so closely together on a branch, we were unable to separately measure the photosynthetic rate, transpiration, and water potential of new- versus old-growth foliage. High humidity in the greenhouse during July prevented us from taking measurements at that time point.

Insect Measurements. Starting in early June, we measured adelgid density on both new- and old-growth foliage on two randomly selected branches per tree. On each branch, the length of new- and old-growth foliage was recorded and the density of both unsettled and dead (first-instar adelgids, distinguishable by their black coloration and lack of woolly covering) and mature (older adelgids, distinguishable by their larger size and white woolly covering) adelgid were counted. Density counts were taken every three weeks from early June

through the end of the experiment; data from the two sampled branches was averaged to determine the number of settled and mature adelgids per cm new- and old-growth foliage per tree. The experiment ended when all of the progrediens-generation adelgids had either matured and reproduced or died.

Statistical Analysis. Because new- and old-growth foliage responded differently to our treatments, we analyzed them separately. We analyzed the effect of light and water on *A. tsugae* settlement using a two-way ANOVA, and assessed changes in *A. tsugae* density and survival over time using a two-way rm-ANOVA. Initial plant height was included in analyses to account for pre-existing size differences. We analyzed the effect of light, water, and *A. tsugae* infestation over time on relative water content (“RWC”), photosynthetic rate, transpiration, and water potential using a three-way rm-ANOVA. RWC was measured for both new- and old-growth foliage; because photosynthetic rate, transpiration, and water potential could not be measured separately on new- versus old-growth foliage, our analysis of these data does not differentiate between foliage types. All analyses were performed using JMP 10.0.2 (SAS Systems, Durham, NC).

Results

Adelgid Performance. Light affected *A. tsugae* settlement on old-growth but not new-growth foliage (Table 1). Settlement on old-growth foliage was 50% higher in the shade versus light treatment, and 30% higher on water-stressed versus watered plants. The impact of light and water on *A. tsugae* inhabiting old-growth foliage persisted over the course of the experiment: *A. tsugae* density on old-growth foliage averaged 36% higher in the shaded treatment and 18% higher in the water-stressed treatment (Table 1; Fig. 1A and B). Adelgid density on new-growth foliage was not affected by the treatments (Fig. 1B), and survival rates were similar in both old- and new-growth foliage (Table 1; Fig. 2B and D).

Hemlock Performance. There was no main effect of adelgid infestation or water on any of the plant performance variables (Table 2; Figs. 3 and 4). Adelgid infestation did decrease water potential in the watered treatment, but not in the water-stressed treatment (water × adelgid interaction in Table 2; Fig. 4C). In contrast, there was a highly significant main effect of light on the RWC of both new- and old-growth foliage, photosynthetic rate, and water potential. The RWC of foliage from shaded trees was 10–15% higher than for full-sun trees (Fig. 3B). Full-sun trees had higher rates of photosynthesis and transpiration in May, but not in June (time × light interaction in Table 2; Fig. 4A and B). Finally, the water potential of full-sun trees was lower than that of shaded trees throughout the experiment (Fig. 4C).

Discussion

Both light and water availability significantly affected adelgid settlement, but only on old-growth foliage

Table 1. Results of statistical analysis of *A. tsugae*-related variables

Model term	<i>A. tsugae</i> settlement						<i>A. tsugae</i> density						<i>A. tsugae</i> survival					
	Old growth			New growth			Old growth			New growth			Old growth			New growth		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
Light	1,68	6.74	0.012	1,67	0.37	0.54	1,54	5.74	0.020	1,50	0.37	0.55	1,54	2.77	0.102	1,50	1.07	0.31
Water	1,68	4.31	0.042	1,67	1.56	0.22	1,54	4.80	0.033	1,50	1.30	0.26	1,54	2.32	0.134	1,50	0.86	0.36
Light × Water	1,68	0.48	0.490	1,67	1.17	0.28	1,54	1.08	0.303	1,50	0.08	0.78	1,54	1.57	0.216	1,50	0.11	0.74
Initial height	1,68	1.24	0.269	1,67	3.49	0.07	1,54	1.14	0.289	1,50	3.03	0.09	1,54	0.30	0.587	1,50	0.88	0.35
Time	–	–	–	–	–	–	2,53	1.36	0.270	2,49	1.39	0.26	2,53	11.20	<0.001	2,49	2.76	0.07
Time × Light	–	–	–	–	–	–	2,53	2.06	0.139	2,49	0.15	0.86	2,53	0.48	0.622	2,49	2.12	0.13
Time × Water	–	–	–	–	–	–	2,53	0.38	0.680	2,49	1.38	0.26	2,53	1.50	0.232	2,49	0.51	0.61
Time × Light × Water	–	–	–	–	–	–	2,53	0.42	0.660	2,49	0.26	0.78	2,53	0.36	0.701	2,49	0.44	0.65
Time × Initial height	–	–	–	–	–	–	2,53	3.25	0.047	2,49	0.89	0.42	2,53	0.28	0.756	2,49	0.74	0.48

Values in bold are significant at $P < 0.05$.

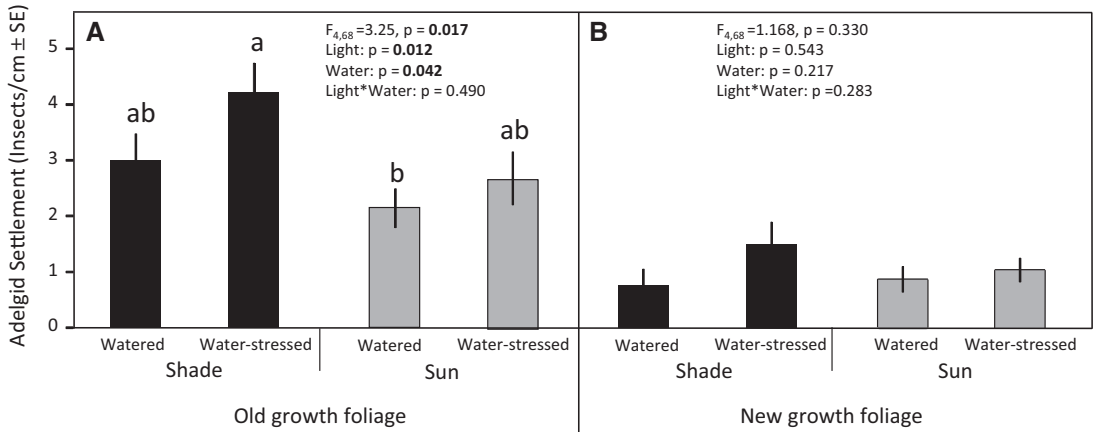


Fig. 1. Settlement densities of adelgids. Darker bars represent shaded treatments, lighter bars are full-sun treatments. (A) Settlement densities (\pm SE) on old growth. (B) Settlement densities (\pm SE) on new growth. These data are represented in the 6 June time point in Fig. 2A and C.

(where the majority of crawlers settled; Fig. 1A and B). Because *A. tsugae* survival was consistent across treatments (Table 1), variation in *A. tsugae* settlement yielded differences in *A. tsugae* density over the course of the experiment. Our results thus suggest that variation in these abiotic factors can substantially alter adelgid population dynamics and may lead to especially high-density infestations in shaded and xeric conditions. Because higher adelgid densities should lead to more rapid hemlock decline, our results may help explain why trees growing in low-light understory conditions or in drier areas appear particularly hard-hit by this pest.

While we expected that old- and new-growth foliage would differ in adelgid settlement, the magnitude of the difference in adelgid settlement was striking. While the mobile crawlers were found in high densities on both types of foliage, they were considered “settled” only when they began producing wool; a large fraction of crawlers on new-growth foliage never progressed to this stage. As a result, there was often a clear line between settled, wool-producing insects on old growth and black wool-free insects on new growth. Adelgids typically insert their stylet bundle proximal to the plant and the needle abscission site (Young et al. 1995, Oten et al. 2014). When settling on the current year’s growth

(e.g., the new growth in our study), however, adelgids will insert their stylet bundle distal to the plant. This may result in needle abscission, or the insect withdrawing its stylet bundle (Young et al. 1995); either outcome would likely prove fatal to vulnerable crawlers. Although insects may be drawn to newly produced foliage, the “green” and highly flexible nature of this tissue may interfere with long-term stylet placement or favor needle abscission. Our result is thus consistent with previous work showing that while the sistens generation prefers the current year’s growth (McClure 1991), the progrediens generation (which we examined) settle preferentially on the previous year’s growth.

Adelgid settlement on old-growth foliage was 50% higher on shaded versus full-sun trees, a result that appears consistent with work on trees growing in forested habitats. Research into the vertical stratification of adelgids found higher densities on lower branches than in the sunnier upper canopy (Evans and Gregoire 2007). One reason for this may be that wool-free adelgids are very fragile and prone to desiccation. Studies have shown that adelgid are very susceptible to cold temperatures (Skinner et al. 2003), and ongoing research suggests that even brief periods of intense summer heat can substantially decrease adelgid survival

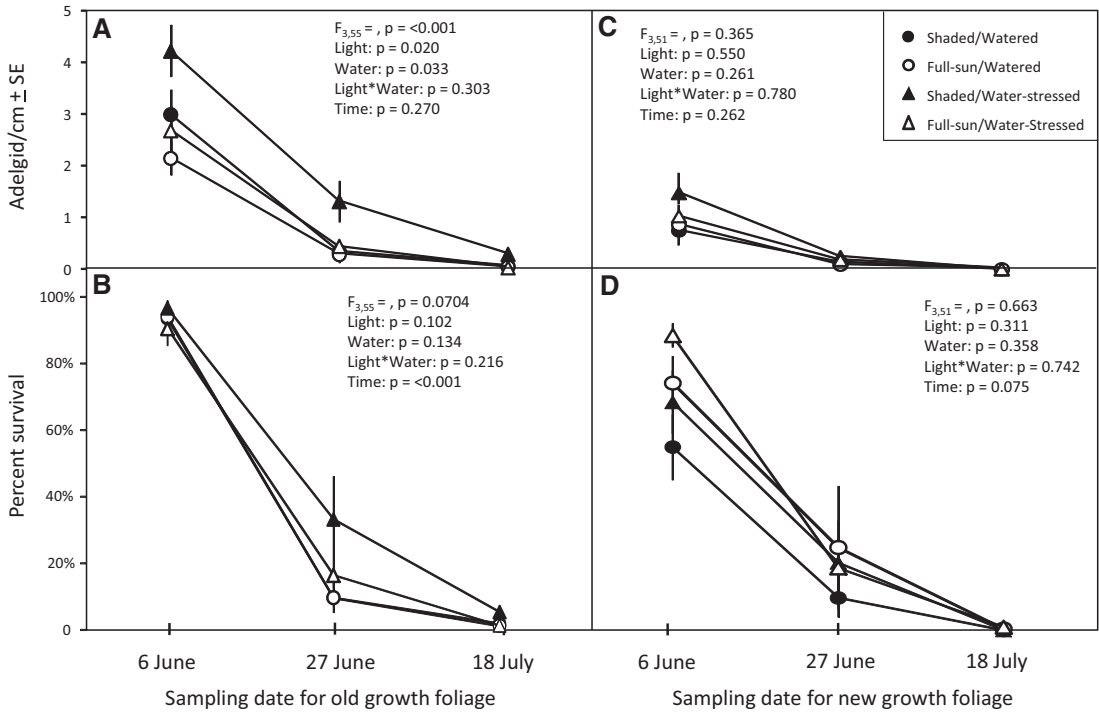


Fig. 2. Density and survival of adelgids throughout the summer. Solid markers represent shaded treatments, open markers represent full-sun treatments, circles represent watered trees, and triangles represent water-stressed trees. (A) Density of adelgid (\pm SE) on old growth. (B) Percent survival of adelgid (\pm SE) on old growth. (C) Density of adelgid (\pm SE) on new growth. (D) Percent survival of adelgid (\pm SE) on new growth. Data from the first time point (6 June) in (A) and (C) are represented in Fig 1 A and B, to help enhance the interpretation of the settlement results.

Table 2. Results of statistical analysis of hemlock-related variables

Model term	Relative water content						Photosynthesis rate			Transpiration rate			Water potential		
	Old growth			New growth			df	F	P	df	F	P	df	F	P
	df	F	P	df	F	P									
Light	1,114	35.63	<0.001	1,110	352.20	<0.001	1,129	5.03	0.03	1,129	1.73	0.19	1,114	5.97	0.02
Water	1,114	0.00	0.945	1,110	0.43	0.51	1,129	0.14	0.71	1,129	0.17	0.66	1,114	3.50	0.06
Adelgid	1,114	1.06	0.306	1,110	0.09	0.76	1,129	0.77	0.38	1,129	1.73	0.19	1,114	2.43	0.12
Light \times Water	1,114	2.80	0.097	1,110	15.11	<0.001	1,129	0.03	0.85	1,129	0.52	0.47	1,114	0.03	0.85
Light \times Adelgid	1,114	0.02	0.885	1,110	0.01	0.98	1,129	0.04	0.85	1,129	0.01	0.91	1,114	2.40	0.12
Water \times Adelgid	1,114	0.01	0.951	1,110	0.51	0.48	1,129	0.04	0.84	1,129	1.11	0.29	1,114	5.96	0.02
Time	1,114	10.12	0.002	2,109	78.64	<0.001	1,129	0.17	0.68	1,129	124.53	<0.001	2,113	71.14	<0.001
Time \times Light	1,114	1.03	0.313	2,109	1.89	0.16	1,129	4.61	0.03	1,129	4.99	0.03	2,113	1.37	0.26
Time \times Water	1,114	0.37	0.545	2,109	0.30	0.74	1,129	1.22	0.17	1,129	1.39	0.24	2,113	1.05	0.35
Time \times Adelgid	1,114	0.28	0.595	2,109	3.04	0.05	1,129	0.71	0.40	1,129	2.16	0.14	2,113	0.77	0.47
Time \times Light \times Water	1,114	1.65	0.202	2,109	4.07	0.02	1,129	1.17	0.28	1,129	0.01	0.92	2,113	0.50	0.61
Time \times Light \times Adelgid	1,114	2.67	0.105	2,109	0.29	0.75	1,129	0.03	0.86	1,129	5.42	0.02	2,113	1.06	0.35
Time \times Water \times Adelgid	1,114	0.04	0.846	2,109	2.59	0.08	1,129	0.15	0.70	1,129	0.23	0.63	2,113	1.43	0.24

Values in bold are significant at $P < 0.05$.

(Sussky and Elkinton 2014). Furthermore, sun stress on a shade-adapted plant can cause the breakdown of photosystems, proteins, and nucleic acids (Demmig-Adams and Adams III 1992). These light-stress-induced problems cause the sap-feeding azalea lace bug *Stephanitis pyrioides* Scott (Heteroptera: Tingidae) to do better on shaded plants rather than ones grown in full sun (Trumbule and Denno 1995). Although we did not test for the breakdown of photosystems,

proteins, or nucleic acids, such changes could have resulted in reduced adelgid settlement on full-sun trees. Further studies would be useful to determine whether adelgid crawlers exhibit negative phototactic behavior that causes them to move away from the sun, or if the sunlight itself is killing the insects once they settle.

The fact that adelgid settlement was 38% higher on water-stressed trees suggests that *A. tsugae* may

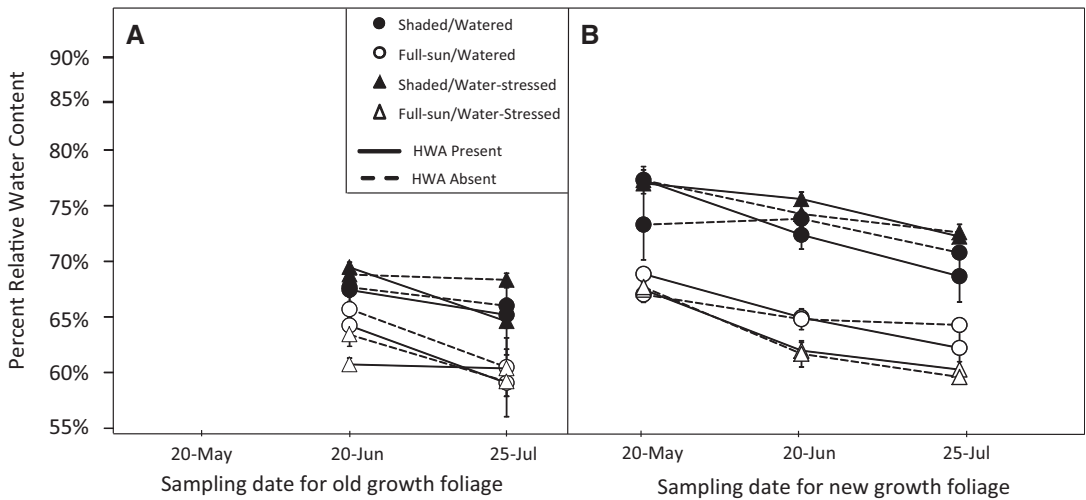


Fig. 3. Percent relative water content (\pm SE) in needles on old growth (A) and new growth (B). The legend is the same as fig. 1 for abiotic treatments; solid lines connect adelgid (“HWA”)–present treatments and dashed lines connect adelgid-absent treatments. Light availability significantly affected percent relative water content in new- and old-growth foliage (B). The percent relative water content of old-growth foliage was 10–15% higher in shaded trees than full-sun trees. However, adelgids had no effect on percent relative water content.

respond positively to some aspects of plant stress. Because plant morphology, physiology, and water use can be negatively impacted by soil drought (Sperry et al. 2002), higher settlement densities on water-stressed trees is consistent with the hypothesis that abiotic stress renders some plants more susceptible to herbivores (White 1984). Our findings are also consistent with work showing that piercing–sucking insects such as adelgids have higher relative growth rates and reproductive potential on stressed plants (Koricheva et al. 1998). The adelgid may differ from otherwise similar insects, however, in its ability to substantially alter water relations within the tree. A recent field study found that adelgid presence lowered water potential by 45% relative to uninfested trees (Gonda-King et al. 2014). This finding is consistent with another showing that adelgid decreases water potential and hydraulic conductivity, and results in the production of wood with no constitutive xylem ducts (Domec et al. 2013). This large impact on water relations within the tree may result from the adelgid altering the tree to be on an even more suitable host, and suggests that the adelgid may do better at lower hydraulic conductivity and water potentials.

Despite high rates of *A. tsugae* settlement, the adelgid did not directly impact any of our plant physiological measurements. This was surprising because herbivory is well-known to alter plant morphology and physiology (Karban and Baldwin 2007), and adelgids have been shown to affect hemlock water potential, photosynthesis, stomatal conductance, and tree water use (Domec et al. 2013, Gonda-King et al. 2014). Adelgids did decrease water potential, but only in the well-watered treatment: there was no similar effect in the water-stressed treatment (Table 2; Fig. 4C). Because adelgids are known to cause water stress, we

would have expected them to exacerbate the decrease in water potential for water-stressed trees. Instead, adelgids significantly altered RWC in the watered, but not the water-stressed, treatment. The fact that they did not alter the water potential of the water-stressed trees may reflect the fact that while those trees are already physiologically suitable for the insects, the watered trees are not (meaning that adelgids need to induce water stress to improve their suitability). This may help explain why adelgids have a greater impact on well-watered trees compared with those trees already experiencing water stress.

Although some of our plant physiology results appear at odds with those of earlier studies (Domec et al. 2013, Gonda-King et al. 2014), it is more likely that our short-term greenhouse experiment was not long enough to detect adelgid-induced physiological changes within the plant. While greenhouse studies provide the ability to precisely control soil moisture levels and other abiotic factors, the environment was unavoidably different from what hemlocks would normally encounter. While we carefully regulated greenhouse temperatures and rotated the trees biweekly within the greenhouse, we cannot reject the hypothesis that the trees experienced some greenhouse-related effects. While we are confident in our results, one future experiment might involve repeating this work in a natural setting.

The short-term nature of our experiment is also likely responsible for the fact that there was no direct impact of our water manipulation on any of our physiology measurements. Furthermore, hemlocks are shade-tolerant trees and the greenhouse conditions may have made the physiological impacts more uniform across trees. In contrast, light availability had a substantial effect on hemlock physiology, but there was no interaction between this factor and adelgid presence

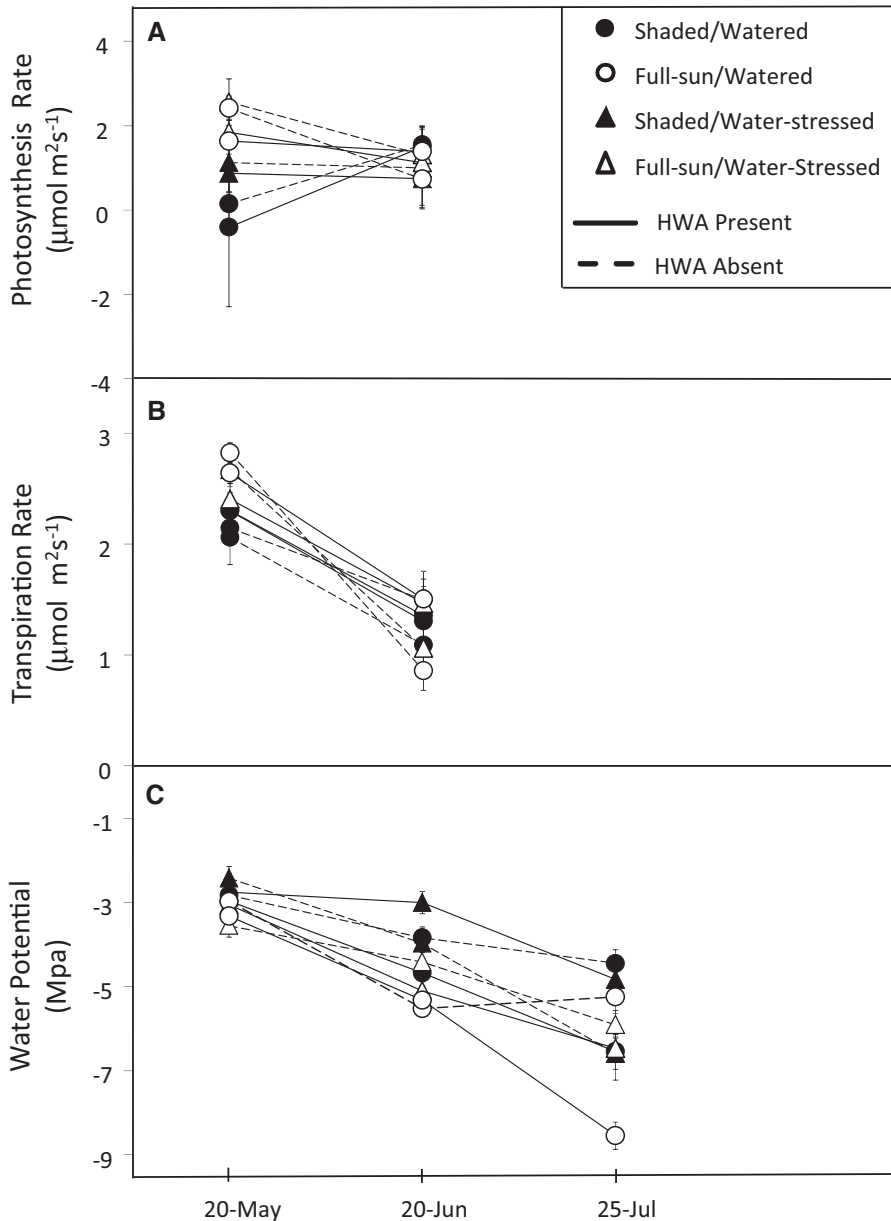


Fig. 4. (A) Photosynthesis (\pm SE), (B) transpiration (\pm SE), and (C) water potential (\pm SE) of trees after adelgid were added. Legend is the same as in fig 3. Photosynthetic rate and water potential were significantly affected by light. There was also a time \times light interaction because photosynthetic rates and transpiration were higher for full-sun trees in May but not in June. Water potential was lower for full-sun trees throughout the experiment. Adelgids had no effect on photosynthesis, transpiration, or water potential.

(Table 2). The impact of light is unsurprising given its importance to plant growth (Pacala et al. 1994), and as full-sun trees had 4.5 \times more light exposure than shaded trees, we expected to see large physiological differences.

In conclusion, adelgid settlement was higher on the old growth of shaded and water-stressed trees, but their survival was not altered by foliage age or either abiotic factor. Although we expected the adelgid to exacerbate the impact of shading and drought stress, we found no

evidence that this was the case; this may, however, be the result of the relatively short duration of our study. While we anticipated that adelgids would settle more readily on new-growth foliage, their observed preference for old-growth foliage agrees with the work of McClure (1991), who found the progrediens generation preferred settling on old-growth foliage; this may be due to the fact that the newest-growth tissue available to settling progrediens is so "green" that it interferes with stylet bundle insertion and feeding. From a

management perspective, the fact that adelgid settlement was so much greater on shaded trees suggests that silvicultural interventions to increase light levels for particularly valuable trees might prove worthwhile. The fact that settlement was higher on water-stressed versus well-watered trees also suggests a possible mechanism for the findings of large-scale surveys that reveal higher rates of adelgid-related mortality for trees growing in xeric conditions (Preisser et al. 2008). From a broader perspective, our results also suggest that management strategies targeting adelgid settlement may prove fruitful in slowing or reducing pest-related hemlock mortality.

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